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Evaluation of Sterilization Performance for Vaporized-Hydrogen-Peroxide-Based Sterilizer with Diverse Controlled Parameters

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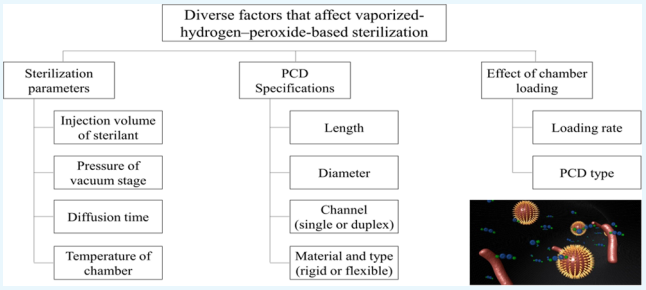
ABSTRACT: Hydrogen-peroxide-based low-temperature sterilization is a new sterilization technology for temperature-dependent medical devices. The effect of the process parameters of hydrogen-peroxide-based sterilizer on the sterilization performance of process challenge devices (PCDs) needs to be investigated. Sterilant amount, operating temperature, vacuum pressure, diffusion time, and chamber loading of the sterilizer on the sterilization performance of PCDs were adjusted. Seven PCDs with various morphologies and material containing biological indicators (BI) (EZTest, *Geobacillus stearothermophilus*) were used to evaluate the sterilization performance. The sterilization success rates of PCDs were 86, 71, and 57% with controlled temperature and pressure, diffusion time, and sterilant volume injection, respectively. The PCD material and structure also obviously affected sterilization performance. The sterilization of PCD A is the least successful for all parameters. Meanwhile, the sterilization of PCD B was influenced by the diffusion time and the sterilant injection volume. PCD B and PCD C were successfully sterilized by controlling the temperature and pressure. The weights and volume of the sterilization loading chamber resulted in a different sterilization performance. Sterilization performances of PCD 1, PCD 2, and PCD 3 were <70, <90, and 100%, respectively. Sterilant volume, sterilant diffusion time, pressure, temperature, PCD types, and chamber loading were proven to be important process parameters of sterilizer that affect the sterilization performance of vaporized-hydrogen-peroxide-based sterilizers.



1. INTRODUCTION

Sterilization forms an important aspect of medical devices, as it eliminates, removes, kills, or deactivates all forms of life and other biological agents existing on healthcare products before their application. Sterilization processes for medical devices are classified into two types of processes: high-temperature and low-temperature processes.[1] High-temperature sterilization is a traditional method that is effective only for heat-stable medical devices. In this regard, the main limitation of autoclave machines is that they are unusable for sterilizing heat-sensitive materials.[2,3] Meanwhile, low-temperature sterilization is essentially required for temperature-dependent medical devices such as complicated minimally invasive surgical products.[2,4] The typical kinds of gaseous chemical sterilants for low-temperature sterilization include ethylene oxide and hydrogen peroxide.[1,5] Since ethylene oxide (EO) is potentially toxic and leaves behind residues on medical devices,[6−8] hydrogen peroxide gas sterilization has been proposed and developed as the new sterilization technology in the market.[9] With regard to vaporized-hydrogen-peroxide-based sterilization, it becomes essential to understand the diverse parameters that affect the sterilization ability to obtain optimal sterilization performance. In this regard, here, we investigate the possible factors that influence the sterilization performance, such as the sterilant characteristics, physical parameters, process challenge device (PCD) specifications, and loading capacity of the chamber utilized for sterilization (Scheme 1).

2. EXPERIMENTAL SECTION

2.1. Test Sterilizer Description. In this study, we used the two following models of hydrogen peroxide sterilizers (Figure 1, Table 1): the LOWTEM Crystal 40R (Lowtem, South Korea) and Steriway 127 (CMtech, South Korea)

The LOWTEM Crystal 40R is a tabletop sterilizer with a chamber capacity of 40 L. This sterilizer utilizes vacuum during the sterilization process, and the sterilant is composed of 59 wt % of hydrogen peroxide. On the other hand, the Steriway 127

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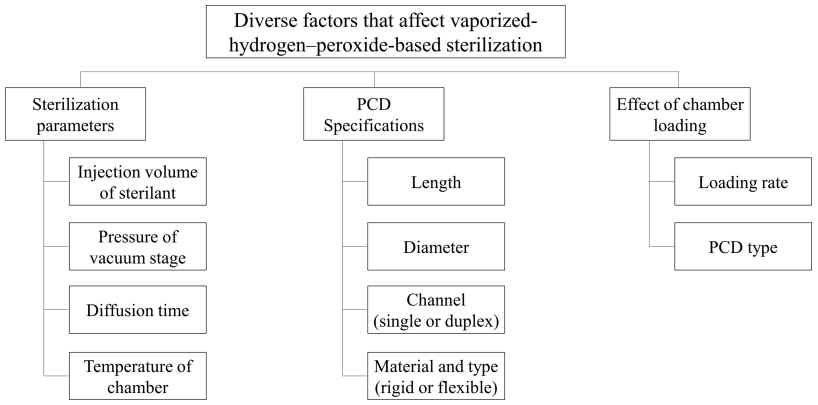
Scheme 1. Overview of Diverse Factors Involved in Sterilization Performance Evaluation



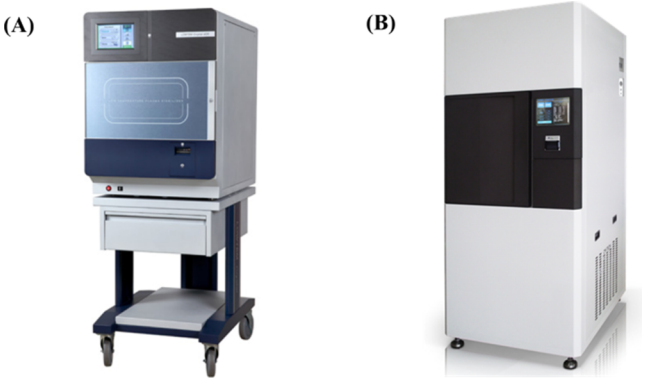


Figure 1. Models of vaporized-hydrogen-peroxide-based sterilizers: (A) LOWTEM Crystal 40R and (B) CMtech Steriway 127.

sterilizer is a stand sterilizer with a chamber capacity of 127 L. This sterilizer also utilizes vacuum during sterilization processing and hydrogen peroxide and ozone as the sterilant. The sterilant is composed of more than 95 wt %/90 mol % of hydrogen peroxide solution (achieved by a concentration process).

2.2. Test Microorganism. *Geobacillus stearothermophilus* (ATCC 7953) is a Gram-positive aerobic bacillus that is found in high-temperature (55−60 °C) environments. In our study, the microorganism was mainly used as an indicator organism to demonstrate the sterilization performance of the vaporized-hydrogen-peroxide-based sterilizers.[9,10] The test microorganism was used as a form of a self-contained biological indicator (SCBI) (EZTest, Mesa Labs, Bozeman, MT), wherein a microorganism population of about 2.1×10^6 spores is contained in a stainless steel carrier with the diameter of 7.16 mm.

2.3. Sterilization Performance Depending on the Injection Volume of the Sterilant. The vaporized-hydrogen-peroxide-based sterilizer (LOWTEM Crystal 40R, Lowtem Co., Ltd., South Korea) was tested with a reduced injected volume of sterilant (half the normal volume) in the half-cycle mode (Table 2). We utilized seven different PCDs based on the type and size, and the SCBI (EZTest, *G. stearothermophilus*, population of 2.1×10^6) was located inside the PCDs to evaluate the sterilization performance (Table 3). After sterilization was performed over a half-cycle, the BIs were incubated at 55 °C for 24 h. The success of sterilization was demonstrated by the color change of the BI; purple corresponded to successful sterilization, while yellow indicated the failure of sterilization because of bacterial growth.

2.4. Sterilization Performance as a Function of Process Parameters (Temperature, Pressure, Diffusion Time). The operating conditions of Lowtem Crystal 40R were varied from the normal operating conditions to evaluate the sterilization results as a function of the process parameters. The temperature in the sterilizer chamber was reduced to 35 from 55 °C via software control. Further, the pressure of the sterilization chamber was increased during fumigation more than that of the normal operating condition (from 1 to 10 mmHg). Thus, a lesser degree of vacuum than in the normal case was realized. The diffusion time was varied from 180 to 90 s (Table 2). The effect of each parameter on the sterilization process was estimated in the half-cycle mode, and one parameter was varied while the others were maintained at standard values. Successful sterilization was confirmed with the color change of BIs, which were located inside the PCDs (Table 3) during the process of sterilization (Figure 2).[11]

Table 1. Specifications of the Sterilizers

Sterilizer	Lowtem Crystal 40R	Steriway 127
Sterilization methods	Vaporized hydrogen peroxide	Vaporized hydrogen peroxide, ozone
Sterilant	Liquid hydrogen peroxide	Liquid hydrogen peroxide, ozone gas
Dimensions	530 mm × 740 mm × 790 mm	850 mm × 1120 mm ×1900 mm
Chamber	300 mm × 220 mm × 640 mm, 40 L, rectangular	380 mm × 700 mm × 480 mm, 127 L, rectangular
Cycle temperature	50 ± 5 °C	<60 °C
Cycle time	Quick: 25 ± 5 min	Standard: 28 ± 2 min
	Standard: 35 ± 5 min	Hybrid: 44 ± 2 min
	Special: 45 ± 5 min	Surface: 33 ± 2 min
Electrical requirement	AC 110, 220 V 50, 60 Hz	380 V, 60 Hz, 3 Phase, 5 kVA

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Table 2. Methods of Variation of Sterilization Parameters

parameter	temperature	pressure	diffusion time	injection volume of sterilant
Variation	Reduction of temperature sterilization chamber, door, injection (vaporizing) valve from 55 to 35 °C.	Sterilization chamber pressure was increased during fumigation from 1 to 10 mmHg.	Fumigation time was reduced from 180 to 90 s.	Sterilant volume was reduced from 1.4 to 0.7 mL
Method	Via software control of sterilizer	Sterilant was not concentrated, and a weaker degree of vacuum relative to the normal process was utilized.	Via software control of sterilizer	Sterilant volume was reduced from 1.4 to 0.7 mL

Table 3. Specifications of PCDs for Process Parameters Tests[a]

name	materials	diameter (mm)	length (mm)	channel
PCD A	PTFE	2	4000	single
PCD B*	PTFE	2	1500	single
PCD C**	PTFE	2	1500	single
PCD D	PTFE	1	1000	single
PCD E	PTFE	1	10 000	duplex
PCD F	stainless steel	1	2000	duplex
PCD G	stainless steel	1	600	duplex

[a]PCDs B* and C** differ in terms of the capsular body material (stainless steel and poly(ethylene), respectively).

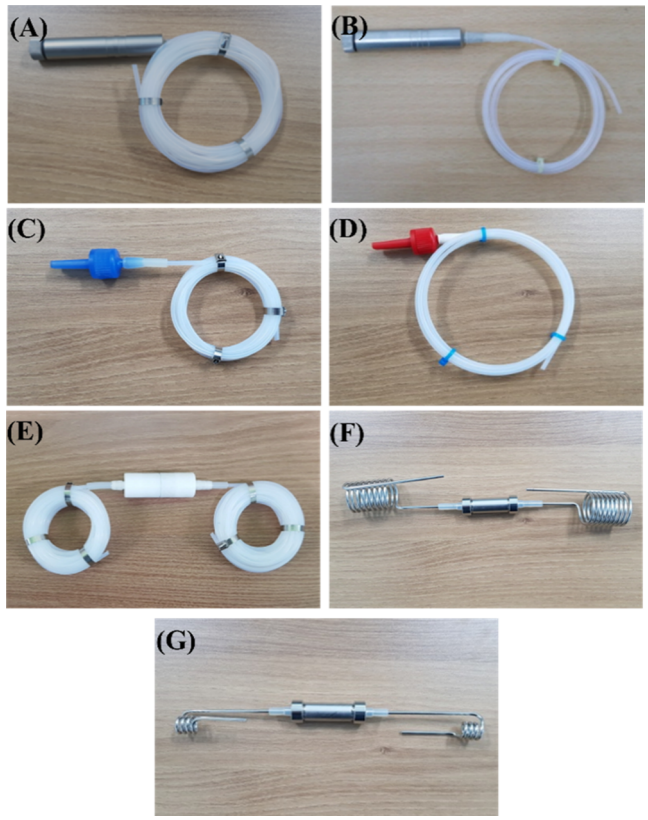

Figure 2. Various shapes and configurations of PCDs utilized for process parameters. The PCD specifications are listed in Table 3. (A) PCD A, (B) PCD B, (C) PCD C, (D) PCD D, (E) PCD E, (F) PCD F, and (G) PCD G.

2.5. Sterilization Performance Dependence on PCD Specifications. PCDs of diverse lengths, diameters, materials, and the number of channels were designed and utilized to estimate the impact of the sterilization performance of vaporized-hydrogen-peroxide-based sterilization as a function of the process parameters. The various parametric changes of temperature, pressure, injection volume of sterilant, and diffusion control in the equipment were evaluated using diverse PCD specifications to determine and test the most difficult types of PCDs for sterilization.[12] The PCDs were prepared using different materials, tube diameters and lengths, and one-way or two-way channels (Table 3).

2.6. Effect of Loading in the Sterilization Chamber on Sterilization Efficacy. The sterilization performances need to be evaluated for actual situations, such as that in hospitals. Consequently, Steriway 127 was evaluated for both PCDs

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(Table 4) and surgical instruments such as forceps, scissors, and 10 kg clamps in the full cycle mode. Further, the

Table 4. Specifications of Process Challenge Devices for Chamber Loading Tests

Name	Materials	Diameter (mm)	Length (mm)	Channel
PCD 1	Stainless steel	0.7	500	Duplex
PCD 2	Stainless steel	1.0	500	Duplex
PCD 3	PFA	0.5	850	Duplex

sterilization efficacy for the maximum possible chamber loading was also tested. As regards the PCDs, stainless-steel-based rigid PCDs were designed with a length of 500 mm and diverse diameters (0.7, 1.0 mm/Figure 3A,B), and flexible

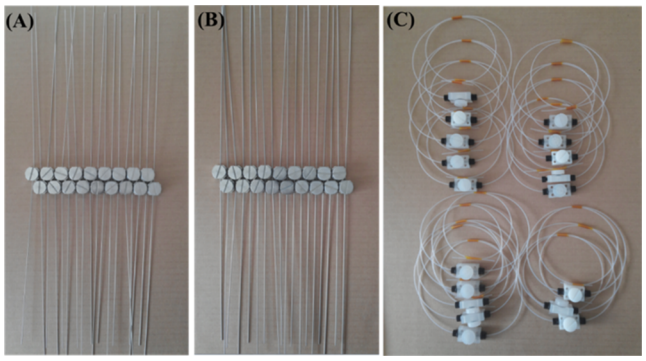

Figure 3. Various shapes and configurations of PCDs for chamber loading. The PCD specifications are listed in Table 4. (A) PCD 1, (B) PCD 2, and (C) PCD 3.

PCDs (perfluoroalkoxy alkane, PFA) were fabricated with a length of 850 mm and diameter of 0.5 mm (Figure 3C). Successful sterilization was confirmed with the color change of BIs, which were located inside the PCDs during the process of sterilization.

3. RESULTS AND DISCUSSION

3.1. Sterilization Performance as a Function of Process Parameters (Diffusion Time, Pressure, Temperature) and Injected Volume of Sterilant. To demonstrate the effect of process parameters on sterilization, we changed the Lowtem Crystal 40R settings as follows: the temperature was reduced from 55 to 35°C, the pressure was increased to reduce the degree of vacuum in the chamber, and the chamber diffusion time was reduced from 180 to 90 s (Table 2). In addition, we established the sterilant volume test condition as corresponding to half the volume of the sterilant normally injected into the Lowtem Crystal 40R. The PCDs for these process parameters and reduced sterilant injection were selected to observe the sterilization performance with seven types of diverse PCDs (Table 3). After the incubation of the BIs of the PCDs, we calculated the success rates of sterilization by changing the parameters (Figure 4). The controls of PCDs were found to be successfully 100% sterilized under the general conditions without any parameter changes. However, the sterilization success rates were affected by changes in the process parameters: the PCD success rate was 86% with the controlled temperature and pressure. Variation in the diffusion time afforded a success rate of about 71% upon reducing the fumigation time. The most important parameter was the

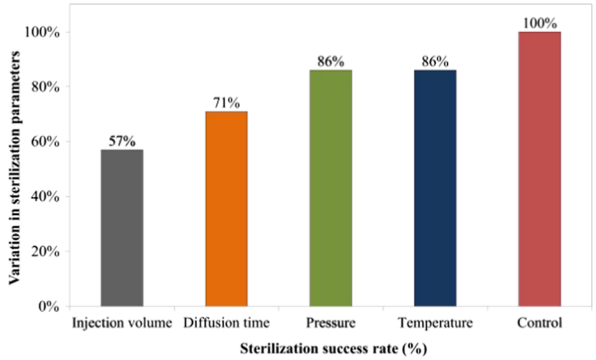

Figure 4. Sterilization success rate as a function of varying sterilization parameters.

injected volume of the sterilant, whose variation afforded a 57% sterilization success rate. Other conditions are maintained general except each parameter.

3.2. Sterilization Performance Depending on PCD Specifications under Various Sterilization Conditions. A PCD is designed to represent the worst-case sterilization scenario to demonstrate the efficacy of the sterilization performance.[9,13,14] Medical devices with long and narrow lumens, when subjected to low-temperature-hydrogen-gas-based sterilization, suffer from the problem that the sterilant cannot easily penetrate the lumen. Thus, the chosen PCDs have to be positioned in the most "inaccessible" chamber locations to confirm the efficacy of the sterilization process.[9] To evaluate the sterilization ability of the sterilizer, we considered the following PCD lumen parameters: diameter, length, type of material, and designed shape. In this study, we tested PCDs with lumens of various sizes and designs to determine the worst-case PCD scenarios (Table 3) when process parameters such as temperature, diffusion time, pressure, and sterilant injection volume (Table 2) are varied. As regards the results, we found that PCD A corresponded to the least successful sterilization performance for all parameters; PCD A was composed of Teflon (PTFE, poly(tetrafluoroethylene)), with dimensions of 2 mm × 4000 mm and a single channel. Meanwhile, the sterilization of PCD B was influenced by the diffusion time of the sterilant in the chamber and the injected volume of the sterilant. The control of temperature and pressure enabled the successful sterilization of PCD B, which was composed of Teflon (PTFE) with dimensions of 2 mm × 1500 mm and a single channel. PCD C was similar to PCD B except for the material of the capsular parts (B: stainless steel, C: polyethylene), and the lumen was not sterilized when only the injection volume of the sterilant was reduced (Figure 5A). Further, it was more difficult to sterilize a single-channel sterilant inlet structure than a duplex-channel structure (Figure 5B). In addition, it was more difficult to sterilize PCDs with longer lumen lengths than those with narrow diameters (such as PCD C and D)Table 5.

3.3. Effect of Sterilization Chamber Loading on Sterilization Result. Test loads composed of medical devices such as forceps, scissors, and clamps were placed in the chamber of Steriway 127 to simulate a real-world hospital-like situation. In this phase of the study, we first evaluated and compared the sterilization performance for three types of PCDs in the long-cycle mode in the empty chamber, next test loads of about 10 kg were added to the chamber, and finally, the chamber was maximally filled with the medical products.

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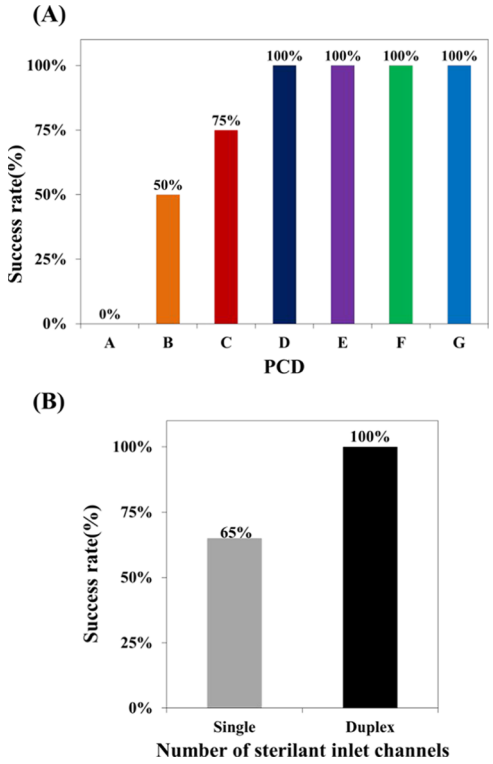

Figure 5. Analysis of the sterilization success result based on Table 5. (A) Sterilization success rates for various process challenge devices (PCDs). The PCD specifications are listed in Table 3. The success rate refers to the number of successful sterilizations relative to the total number of sterilizations. From the figure, the sterilizing difficulty can be understood as A > B > C > D = E = F = G. (B) Sterilization success rate as a function of the number of sterilant inlet channels in the PCD. "Single" indicates that the PCD has only one sterilant inlet, while "duplex" indicates that the PCD has two channels aligned in opposite directions.

Table 5. Sterilization Success Results of Process Challenge Devices Subjected to Controlled Parameter Variation[a]

PCD	Sterilant injection volume	Diffusion time	Pressure	Temperature
A	X	X	X	X
B	X	X	O	O
C	X	O	O	O
D	O	O	O	O
E	O	O	O	O
F	O	O	O	O
G	O	O	O	O

[a]O: sterilization success; X: sterilization failure.

The three PCDs consisted of two rigid types (stainless steel: 0.7 mm × 500 mm, 1.0 mm × 500 mm) and one flexible type (PFA Teflon: 0.5 mm × 850 mm). The PCDs in the empty chamber exhibited a perfect sterilization performance of 100%. After the addition of the 10 kg test loads, the sterilization performance of PCD 1 (0.7 mm × 500 mm) reduced to <80%. Corresponding to the actual hospital-like situation of the chamber containing the maximum number of medical items, the two rigid PCDs exhibited sterilization performances of <70% (PCD 1) and <90% (PCD 2), that is, the different weights and volume loads of the sterilization chamber under the same conditions gave rise to the observed difference in the sterilization performance. PCD 3 was 100% regardless of the chamber loading. Flexible PCDs (perfluoroalkoxy alkane, PFA) were less susceptible when sterilized together with other items (Figure 6).

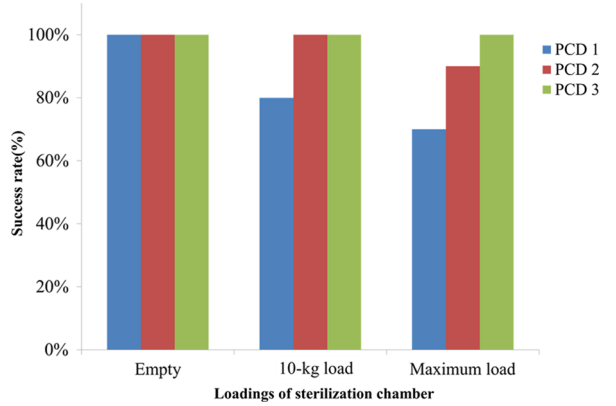

Figure 6. Sterilization success rate for diverse loadings of the sterilization chamber. PCD 1: 0.7 mm diameter, 500 mm length, rigid body, and stainless steel; PCD 2: 1.0 mm diameter, 500 mm length, rigid body, and stainless steel; and PCD 3: 0.5 mm diameter, 850 mm length, flexible body, and PFA.

4. CONCLUSIONS

Several process parameters affect the sterilization performance of vaporized-hydrogen-peroxide-based sterilizers. The volume of the sterilant, the sterilant diffusion time, the pressure control of the sterilizer, the temperature change, the PCD type, and the product loading in the chamber were proved as important process parameters. To improve the sterilization performance of vaporized-hydrogen-peroxide-based sterilizers, it is necessary to determine the optimally controlled process parameters; further, there is a definite need to understand the sterilization characteristics resulting from diverse controlled conditions.

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